

A COMPARATIVE STUDY OF THE CEMENT GLANDS IN SOME BALANID BARNACLES (CIRRIPIEDIA, BALANIDAE)¹

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The structure of the cement glands in Cirripectia was only briefly mentioned by Darwin (1854), Krohn (1859), Koehler (1889), Gruvel (1905a, b) and a few others. Recently, beginning with Thomas (1944), several authors studied various types of epidermal glands in this group of animals (Boucquet-Vedrine and Ovechko, 1960; Utinomi, 1960; Walley, 1967), no one, however, dealt with the specialized glands responsible for the secretion of cement. A detailed histological study by Lacombe (1966) on adult specimens of *Balanus tintinnabulum* called attention to the ectodermal origin of the cement glands. Subsequent histochemical investigations (Lacombe, 1967a) suggested that the intra- and extracellular secretion of the cement glands may be a type of acid mucopolysaccharide. Arvy and Lacombe (1968) and Arvy, Lacombe and Shimony (1968) utilizing histoenzymological techniques showed that the secretion spread in the cell and the extruded secretion within the canal system give a positive reaction for alkaline phosphatase, but the rest of the cement gland cell remains negative. A comparative study of the cement gland in *B. tintinnabulum* and *Lepas anatifera* (Lacombe and Lignori, 1969) suggested that the structure of the cement glands may be less complex in the primitive barnacles, such as the *Lepalidae*.

METHODS

The ovaria with the cement glands of adult specimens of *B. nubilis* and *B. psittacus* were dissected for immediate fixation; in the case of *B. eburneus*, *B. amphitrite* and *B. balanoides* the entire animal was fixed.

The following fixatives were employed: Flemming, Bouin, Susa, Carnoy, Helly, and Altmann's fluid, as well as 10% formaldehyde. The material was embedded in paraffin and sections were cut at 5, 7 and 10 μ .

The sections were stained with Delafield's hematoxylin and Chromotrop 2R, Ehrlich's hematoxylin and Orange G. G., Weigert's hematoxylin and Alcian blue at pH 2.3, Mallory's Azan method with Congo red, Trypan blue with Chromotrop 2R, Aniline blue and Nuclear fast red with Naphthol green. For fine details Heidenhain's iron alum hematoxylin was used, following fixation in Flemming's fluid.

OBSERVATIONS

Typically, as in *B. tintinnabulum* (Lacombe, 1966), the secretory cells which elaborate the cement occur in groups (*B. psittacus*, *B. eburneus* and *B.*

¹ Translated from the Portuguese by Dr. Sophie Jakowska, Universidad Autonoma de Santo Domingo, Dominican Republic.

amphrite) or in rosettes (*B. nubilis*) intermingled with the ovarian follicles. In *B. balanoides*, on the other hand, they are scattered individually among the connective tissues in the basal portion of the animal. The number of cement gland cells appears relatively smaller in *B. balanoides* than in the other species examined.

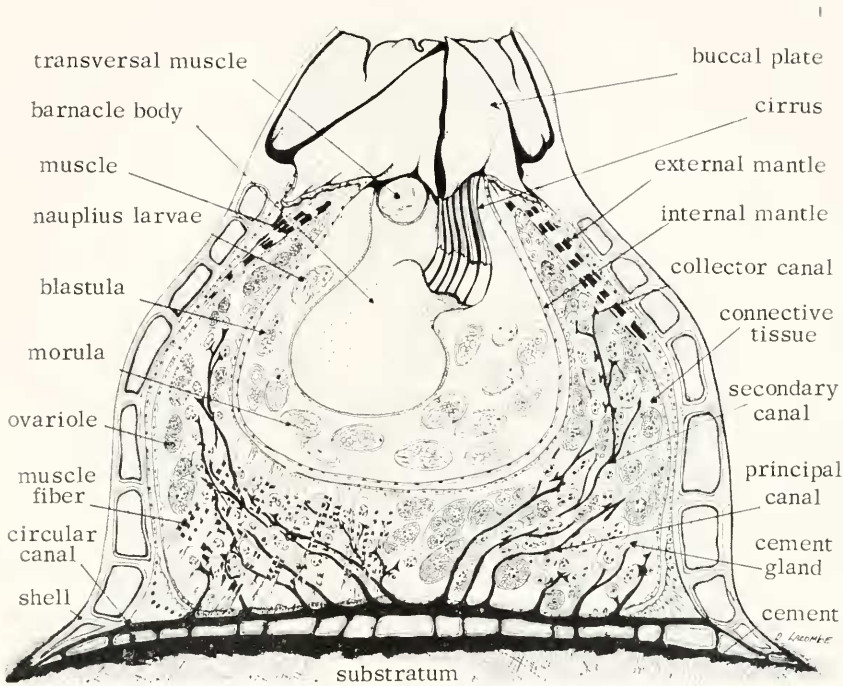


FIGURE 1. Schematic drawing of a longitudinal section in *Balanus* sp., showing the internal anatomy and cement glands with canal system.

Figure 1 is a schematic drawing of the anatomy of the typical balanid barnacle in longitudinal section. The structures include the cement glands among the ovarian follicles, the canal system, through which the cement secretion is conveyed to the basal portion of the animal, and the flat epithelium of the mantle, shown in lateral view.

A. Secretory elements

In *B. nubilis* the cement glands are found in the connective tissue near the ovarian region. In the external zone of the mantle the glands appear to be in the early stages of development (Fig. 2), and consist of small cells differentiating in the walls of the principal and secondary canals (Fig. 3). The young cement glands form groups of 15 to 30 small cells which have dense homogeneous cytoplasm and a central nucleus rich in chromatin.

During their development the gland cells become embedded deeper in the interior of the mantle-chamber and located in the connective tissue near the

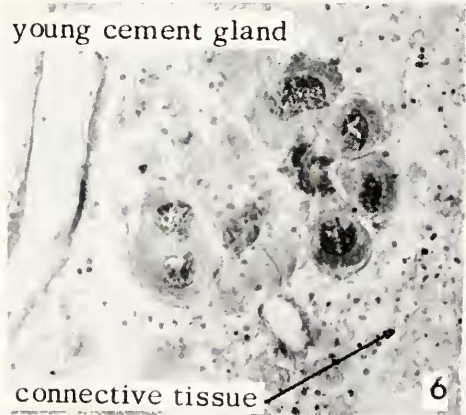
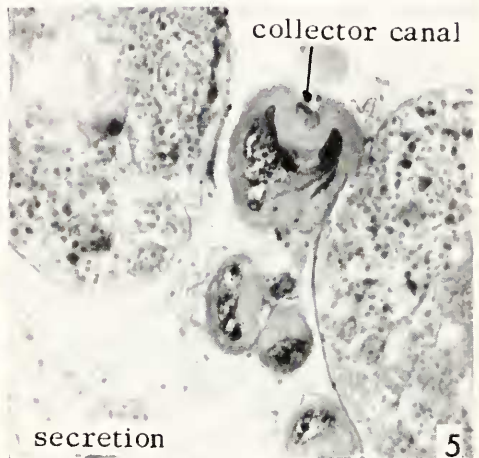
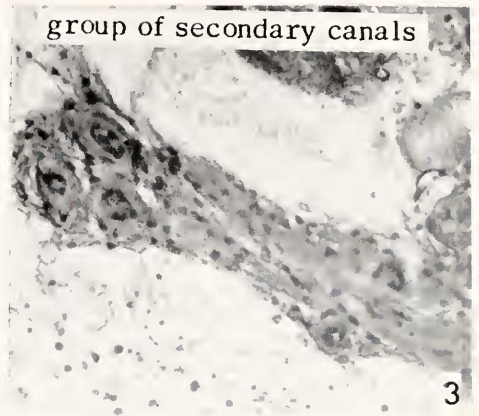
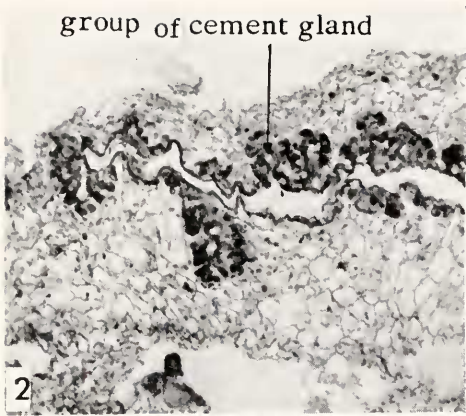


FIGURE 2. *Balanus nubilis*. Longitudinal section through secondary canal with young cement glands in development, Mallory-Azan.

FIGURE 3. Transverse section through principal canals near the hilum region, Mallory-Azan.

FIGURES 4 to 7. Transverse section through the cement glands in different stages; nuclear fast red and naphthol green.

ovarian follicles (Figs. 4 to 7). At this stage the glands begin to elaborate the cement secretion (Fig. 4). The secretion originates in one zone of the cell, the secretory pole, and accumulates in another zone, the storage pole; the latter connects with the collector canal (Fig. 5); that means, from the point of view of genesis of this type of cells, that the apical part of the gland cell stores the secretion and the basal part produces it. Occasionally the cells of the canal walls develop clusters of secretory cells which appear as partial or complete rosettes in histological sections (Fig. 6).

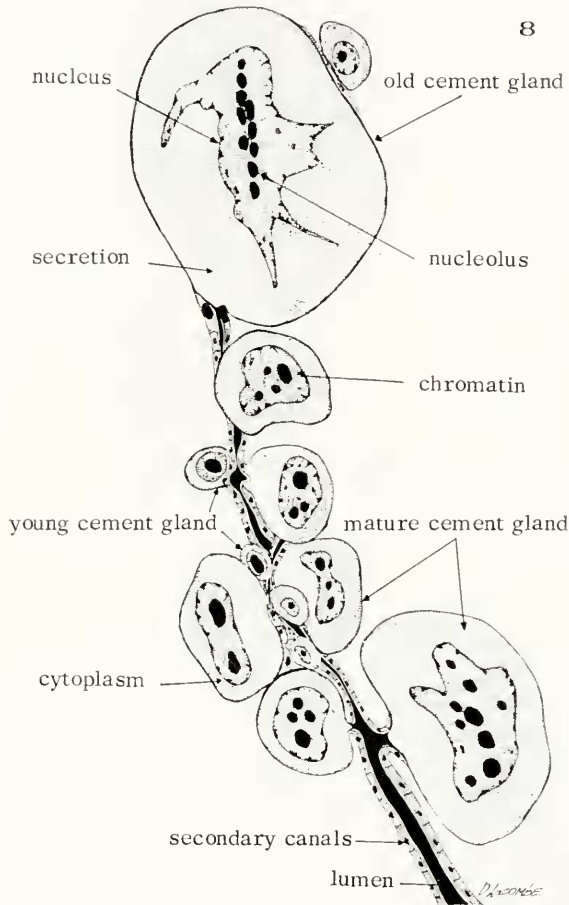


FIGURE 8. Cement glands in *Balanus psittacus* showing different stages of development.

Within such clusters the cement secretion is clearly visible. Young cells forming the clusters have regular, centrally located nuclei, each with two to four nucleoli. In mature cells, the nuclei are polymorphic, with many nucleoli in varied positions. Older cement gland cells have large and more irregular nuclei, and an even larger number of nucleoli; the nuclei are very poor in chromatin indicating an increase of nucleoplasm.

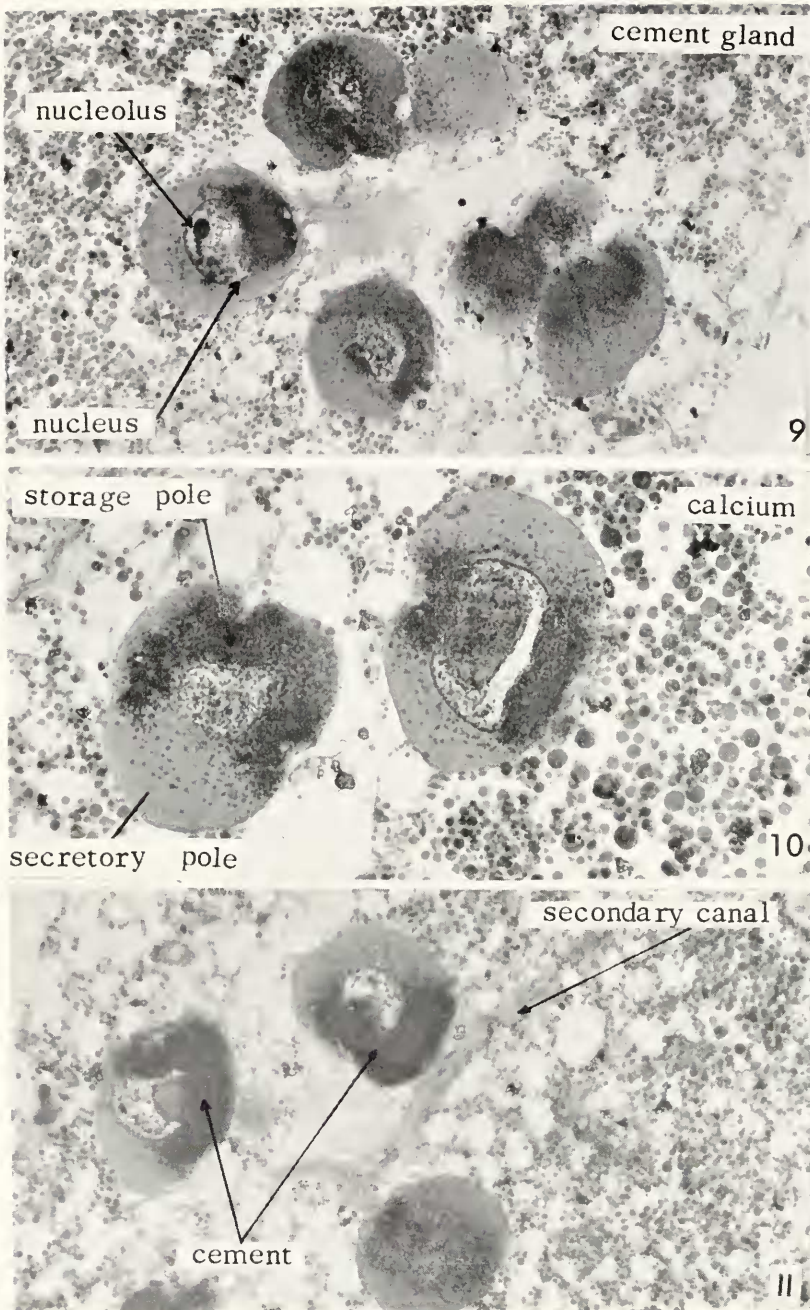


FIGURE 9. *Balanus cburneus*. Intercellular cement secretion; nuclear fast red and Azan method.

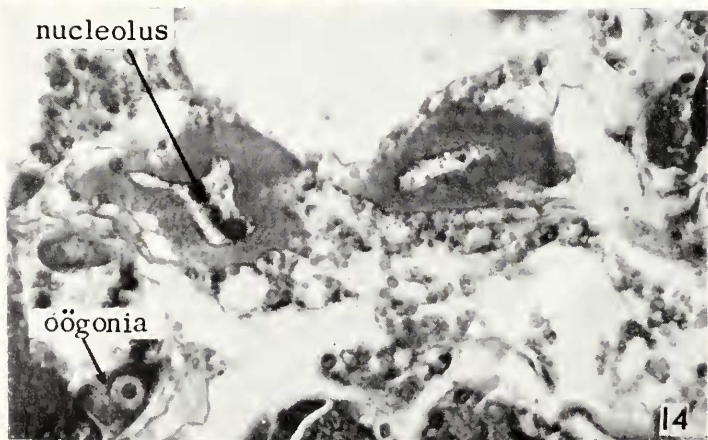
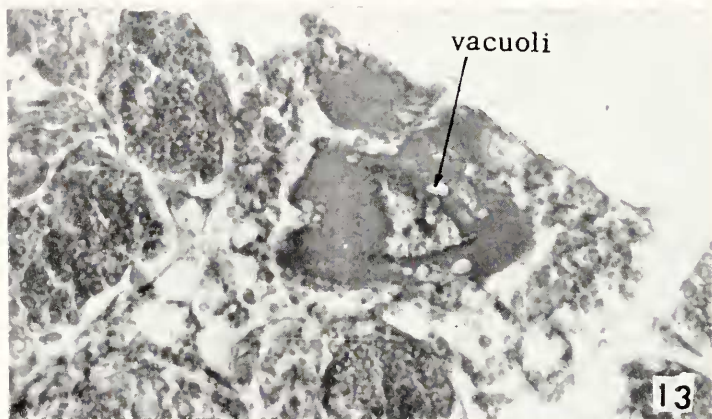
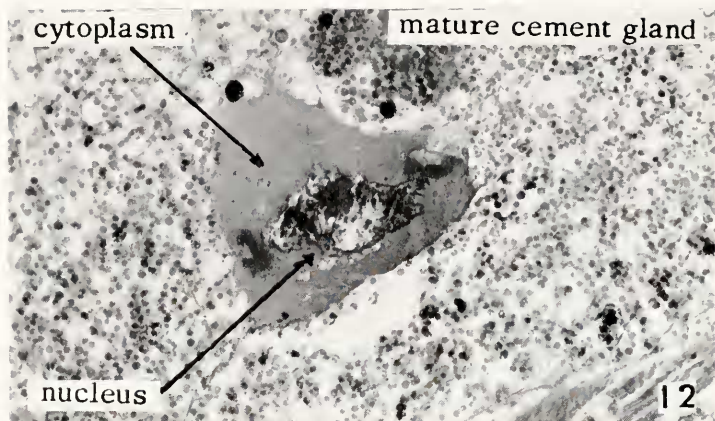
FIGURE 10. Same gland in higher magnification.

FIGURE 11. Longitudinal section through the secondary and collector canals.

In *B. psittacus* the cement glands are much easier to locate than in *B. nubilis*. In both species the epithelium of the mantle occupies a large part of the animal's shell. In the walls of the principal and secondary canals the gland cells grow outward from the lumen. All stages of cell development and maturation may be easily seen (Fig. 8). The cytoplasm of the gland cells exhibits very small vacuoles which increase in size as the cells mature. These vacuoles, full of cement, are distributed throughout the cytoplasm. When stained with the Mallory's Azan method, or with nuclear fast red stain and Alcian blue, the cement gland cells take on a uniform coloration. In *B. psittacus*, an accumulation of the cement at the storage pole, such as in *B. nubilis*, was not observed. The nuclei of the gland cells are large and more polymorphic, with 10-14 nucleoli irregularly distributed through the nucleoplasm. The nuclear chromatin is poor and dispersed. Each group of cement glands is composed of 8 to 14 secretory units or cells in different stages of development.

The gland in *B. eburneus* consists of a few, well-defined cells (Fig. 9). The young gland cells are located very close to the basal plate or the point at which the gland cells begin to differentiate from the canal walls. The mature gland cells are located in the connective tissue among the ovarian follicles. In this species the gland cells do not change in shape and form during the development. The cytoplasm is dense, homogeneous and devoid of vacuoles. The nuclei are constant in form and exhibit very little polymorphism; chromatin is mostly concentrated around the nucleoli, which number two to four. The cement secretion is visible as granular particles scattered in the cytoplasm at the secretory pole of the cell (Fig. 10). At the storage pole the secretion granules are very dense and they leave the cell through a fine membrane, passing into the lumen of the collecting duct (Fig. 11). A similar condition was observed in the cement gland of *B. tintinnabulum* (Lacombe, 1966). At the storage pole of each gland cell of *B. eburneus*, it is seen with the light microscope that the cytoplasm projects into the nucleus as shown in Fig. 10; this condition results in an increase of surface of contact between the secretion and the nuclear elements. In *B. tintinnabulum*, the electron microscope revealed that the endoplasmic reticulum has a different arrangement in this region of contact (Lacombe, 1967b), and suggested a relationship between the nuclear elements and the cement secretion.

The cement glands of *B. balanoides* differ distinctly from those of all the other species described in this paper. In this species the glands (Fig. 12) are located in the basal portion of the animal near the region where the shell increases in size. They never appear as a distinct group of cells or as rosette shaped clusters. The glands consist of single isolated cells and the mature elements are particularly conspicuous in the connective tissue. Young cement glands are rarely seen in adult *B. balanoides*, perhaps due to the fact that the growth of the glands closely parallels that of the animal. Another distinctive feature of these gland cells is the abundance of cytoplasmic vacuoles containing cement secretion (Fig. 13), which gradually increase in size. When many vacuoles containing secretion accumulate, they move toward the cell membrane nearest to the collector canal. As it was observed in *Lepas anatifera* (Lacombe and Liguori, 1969), *B. balanoides* exhibits more than one way of discharging cement secretion into the lumen of the collector canals, and this corresponds to the primitive condition. Figure 12 clearly shows two typical storage and discharge points in a cement gland cell.



FIGURES 12 and 13. *Balanus balanoides*. Cement glands showing the vacuoles and the paths of cement extrusion; Erlich's hematoxylin and chromotrope 2R.

FIGURE 14. Cement glands, with typical nucleoli in the polymorphic nuclei; Heidenhain's Iron hematoxylin.

The cement glands of *B. balanoides* exhibit considerable nuclear polymorphism such as seen in *B. eburneus*. The number of nucleoli varies from four to fourteen and they are clearly defined (Fig. 14). The schematic drawing (Fig. 15) shows different parts of this type of cell.

In *B. amphitrite*, the cement glands closely resemble those of *B. eburneus* in size, localization and form. The glands of *B. amphitrite* are located among the ovarian follicles (Fig. 16) and most frequently near the white muscles. The glands are composed of groups of 6 to 10 cells and are seldom represented by single isolated cells (Fig. 17). During all stages of development the gland cells

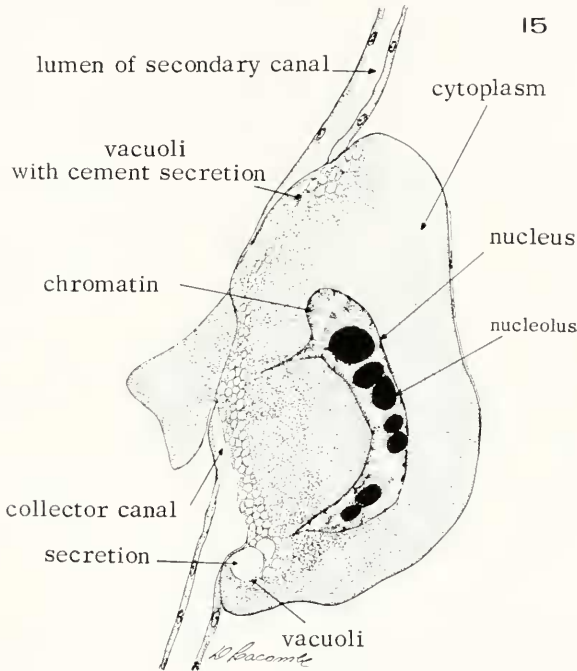
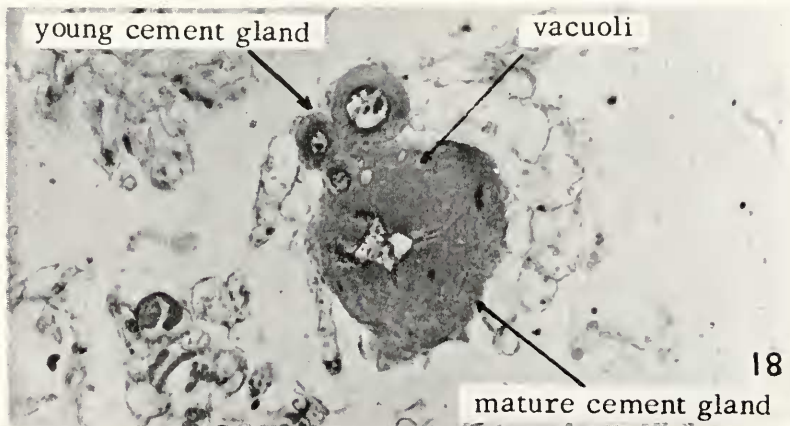
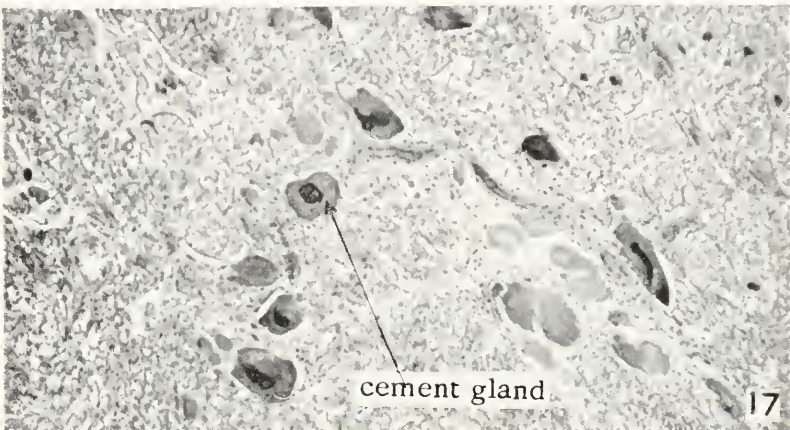
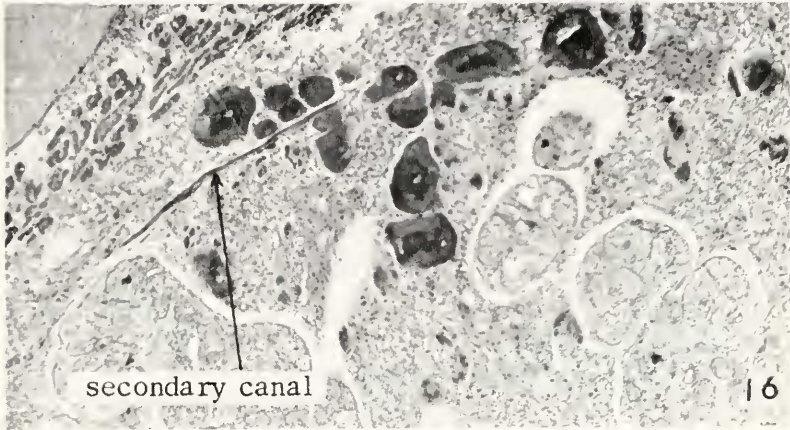


FIGURE 15. Cement gland of *Balanus balanoides*, showing vacuoles rich in cement secretion, nucleoli of different sizes in the polymorphic nuclei.

are regular in form; their cytoplasm is dense and contains small vacuoles near the cell membrane. Usually different stages of developing cells are found growing among the old gland cells (Fig. 18). This condition differs from that observed in *B. balanoides*. The occurrence of differences of cell development simplifies the observation of the cytology of the various cell phases. In young gland cells, the nuclei are round, poor in chromatin and have centrally placed nucleoli. In mature glands, however, the nuclei tend to become polymorphic. The cement secretion of *B. amphitrite* is similar to that of *B. eburneus*, and both species show very distinct storage and secretory poles in the mature gland cells.



FIGURES 16 and 17. *Balanus amphitrite*. Groups of cement gland cells with secondary canals, Delafield's hematoxylin and chromatrope 2R.

FIGURE 18. Different stages of development of cement glands; Erlich's hematoxylin with Congo red and orange G.G.

B. Accessory canal system

The accessory canal system, which distributes the cement, was previously studied in *B. tintinnabulum* (Lacombe, 1966). It originates by progressive invagination of the hypodermal cells of the exterior mantle wall after the cypris larva becomes attached. Thus begins the formation of a complex system consisting of circular, radial and principal canals. The principal canals grow inward and ramify extensively, giving rise to numerous secondary canals. Subsequently, the chitin of the mantle epithelium spreads as a lining over the entire lumen of this canal system. Some cells in the walls of the principal canals or the secondary canals begin to differentiate into the specialized cement glands.

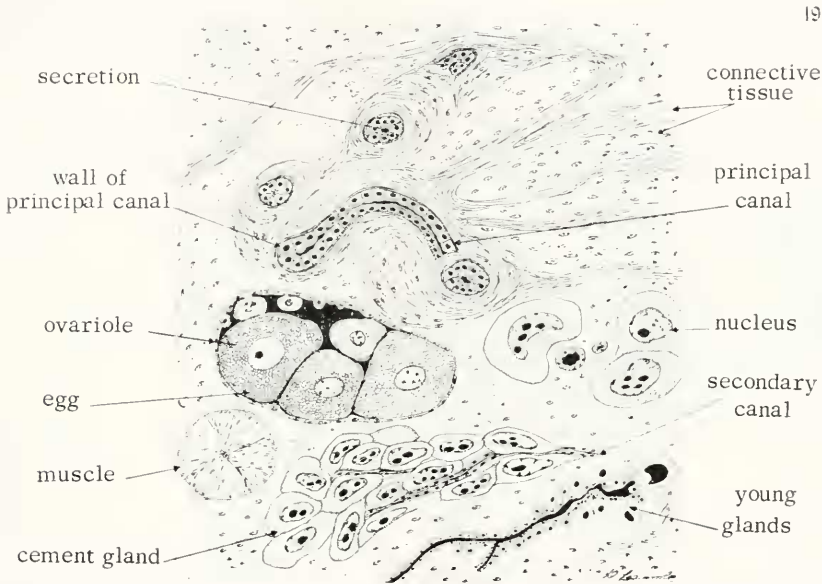


FIGURE 19. Ovarian region in *Balanus nubilis* showing the elastic fibers around the principal and secondary canals, with young, mature and old cement glands and oocytes in development.

The configuration of the canal system varies to some extent in the five species of balanids studied in this paper, but in general the pattern resembles that of *B. tintinnabulum* or that in the schematic drawing of the internal anatomy of a typical barnacle (Fig. 1). The secretion passes through the cell membrane from the vacuoles or from the storage pole into the lumen of the collecting canals, from where it moves on to the secondary and to the principal canals. The principal canals conduct the secretion to the radial and circular canals, from where it spreads out beneath the basal plate by typical outlets.

In *B. nubilis* the secondary canals are narrow and many developmental stages of gland cells may be seen in their epithelium (Fig. 19). The principal canals adjoining the ovarian region have a large diameter and their epithelial cells tend to be larger. The connective tissue fibers are concentrated around the principal

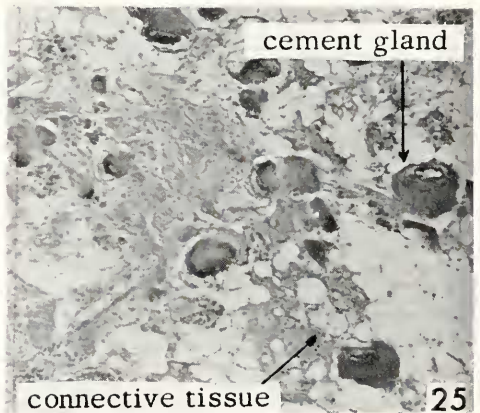
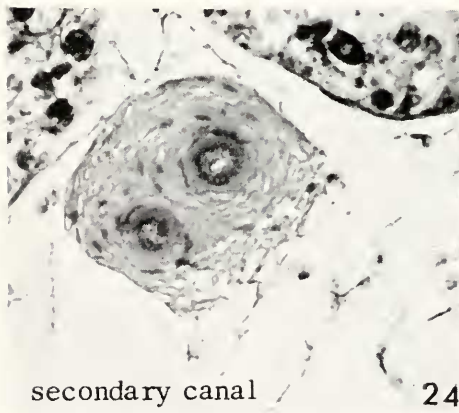
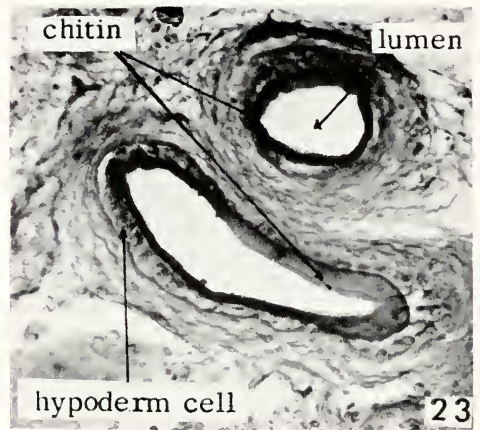
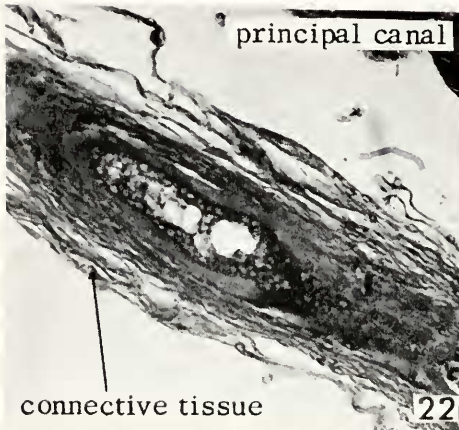
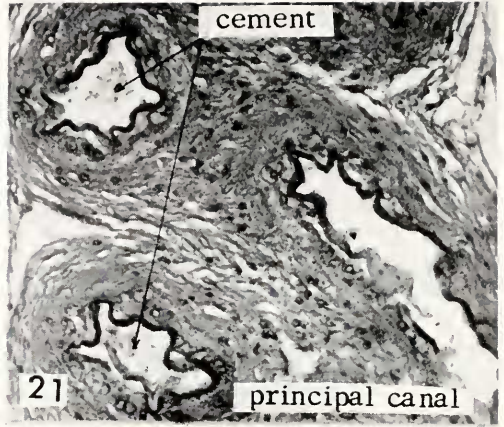
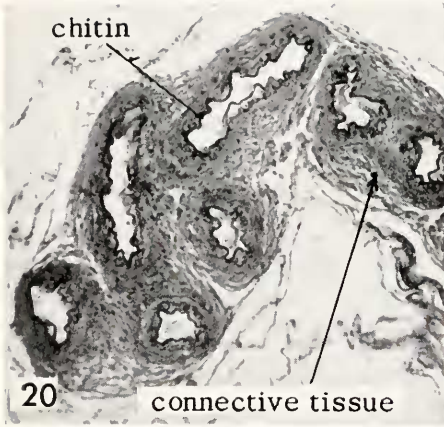


FIGURE 20. *Balanus psittacus*. Principal canals, showing connective tissue fibers, hypodermal cells and chitin; Mallory's Azan method with Congo red.

FIGURE 21. Same section as in Figure 20 showing the cement secretion in the lumen of the principal canal, same stain.

canals and apparently function as a protective covering (Fig. 19). The chitinous layer within the lumen of the principal canals is very difficult to observe, but it becomes evident and is clearly seen with polarized light. In this species, as well as in *B. psittacus*, a hilum appears in regions where the secondary canals join the principal canals.

The hilum is absent in *B. cburneus*, *B. amphitrite* and *B. balanoides*. The principal canals are composed of very simple, flat epithelial cells (Fig. 11) with elongated nuclei; these are rich in chromatin, a fact which indicates that these nuclei are out of function, their nucleoplasm was reduced and their chromatin appears now more concentrated. There are no connective tissue fibers around the principal or secondary canals. The arrangement of the canal system is similar to that of *B. tintinnabulum*.

In *B. psittacus* the hilum is greatly accentuated and in this region all the cement gland cells show discrete secretion. The structure of the principal canals, indicated in Figures 20 to 23, resembles that of *B. nubilis* in which the principal canals have a tortuous course (Fig. 20) and the secondary and the principal canals (Fig. 22 and 24) are surrounded by a large number of connective tissue fibers. The latter feature appears to be typical of the larger species of balanids, such as *B. psittacus* and *B. nubilis*, and it is not observed in the others. In *B. psittacus*, the chitinous layer in the principal canals (Fig. 23) is clearly shown by simple staining with Congo red. The hypodermal cells in this species possess dense cytoplasm and a large, basally situated nucleus, poor in chromatin. Figure 25 shows a general histological view of the hilum region when the cement gland cells and canals are filled with secretion. The secretion in this species is clearly visualized along the entire canal system with simple histological stains. This is not as easily observed with such methods in the other balanids studied where only small granulations appear (Fig. 20–21), but histoenzymological methods permit to demonstrate the cement in those species.

C. Extrusion of the cement secretion

In the serial sections of *B. cburneus*, *B. balanoides* and *B. amphitrite*, stained with Heidenhain's iron alum hematoxylin, the basal muscle of the mantle is easily seen; Gutmann (1960) called attention to these muscles, but did not associate them with the cement glands. These striated muscle fibers extend from the chitinous covering of the external mantle epithelium on the base plate (Fig. 26), to the thin chitin of the inner mantle layer, which covers the body of the barnacle.

The muscle fibers pass through the connective tissue between the ovarian follicles and the cement glands (Fig. 27). These fibers are very distinct, with long tonofibrils in the base plate (Fig. 26) and very short tonofibrils at the mantle side. The striated muscles are closely associated with the cement glands and ovarian follicles as may be seen in cross section (Figs. 27 and 28). Figure 28

FIGURE 22. Principal canal with cement secretion, same stain.

FIGURE 23. Principal canals showing chitin and hypodermal layer and connective tissue transverse section, same stain.

FIGURE 24. Secondary canal near the hilum region, showing distinct connective tissue fibers, transverse section, same stain.

FIGURE 25. General view of the hilum region with cement glands in different stages and the arrangement of the canal system, same stain.

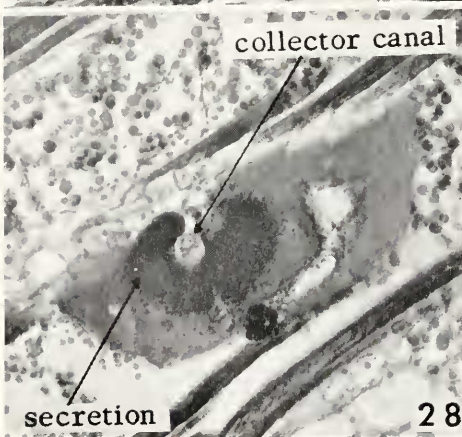
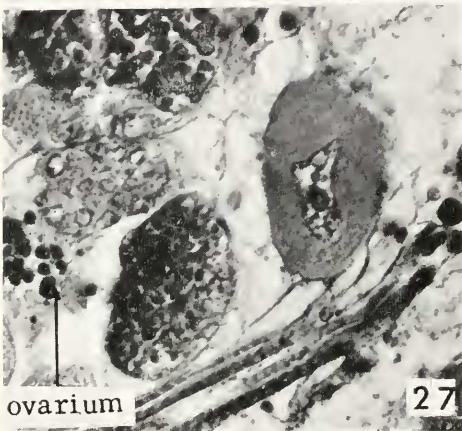
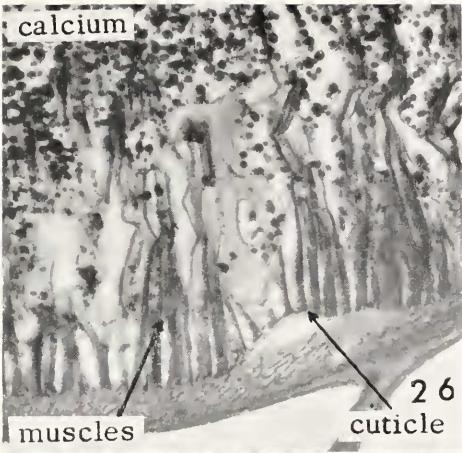


FIGURE 26. Longitudinal section through the base plate of *Balanus cburneus* showing the long tonofibrils and the muscles of the mantle, Heidenhain's iron hematoxylin.

FIGURE 27. The cement gland in *Balanus balanoides* and muscular system of the mantle, same stain.

shows the cement glands of *B. amphitrite*, with the collector canal in cross section next to the storage pole. It might be supposed that this musculature (Figs. 29 and 30) is involved in some way in the movement of the cement secretion from the secondary and principal canals to the basal system. The contraction of these muscle fibers probably compresses the entire ovarian region, which includes the cement glands and the connective tissue, and may cause the cement to move towards the basal canal. Perhaps this contraction helps extrude the cement secretion from the canal system to the substrate in the absence of additional musculature in the walls of the canals. The basic anatomical relationships, seen in Figure 1, suggest the above interpretations.

DISCUSSION

The pattern that seems to emerge from the observations on the Balanids selected for study suggest that the degree of development and differentiation in the cement gland system may be related to the phylogenetic position of the species.

B. balanoides is the species that exhibits a relatively simple pattern: (1) single large secretory cells situated at the base of the animal, away from the ovarian follicles; (2) the secretion scattered throughout the cytoplasm and collected in vacuoles which extend into the collector canal; (3) the absence of elastic fibers around the canals.

B. amphitrite, *B. cburneus* and *B. tintinnabulum* (the last was studied previously by Lacombe, 1966), represent what may be considered as a typical pattern for balanids: 1) large secretory cells, arranged in irregular groups, intermixed with the ovarian follicles; 2) the secretion formed at the secretory pole of the cells and extruded at the opposite pole into the collector conduct without the formation of vacuoles; 3) the absence of elastic fibers in the walls of the canal system.

In contrast to the above species, *B. psittacus* and *B. nubilis* both possess elastic fibers around the canals, and in both, the cement glands are intermixed with the ovarian follicles. In *B. psittacus*, however, the secretory elements consist of irregular groups of small and large cells and in *B. nubilis*, all the cells are small and are arranged in form of rosettes. The secretion is not confined to the vacuoles or to the storage pole, but appears scattered throughout the cytoplasm.

It may be too early to speculate on the significance of these differences observed in the balanids under study. It is generally recognized, however, that *B. balanoides* may represent a primitive form, while the large barnacles, *B. psittacus* and *B. nubilis* may be considered as the more advanced forms of balanids. The morphological differences observed in the cement gland system in these species point to interpretations involving phylogenetic concepts. In addition, one is impressed by the fact that adult specimens of *B. balanoides* exhibit only fully formed cement gland elements, while in the other species one may observe within the same adult specimens the progressive stages of differentiation of these elements.

FIGURE 28. Cement gland and muscle fibers in *Balanus amphitrite*, showing the striated muscles, the storage pole of the gland cell and collector canal in transverse section, same strain.

FIGURE 29. Longitudinal section through the mantle of *B. cburneus*, showing the muscle fibers, same stain.

FIGURE 30. General view of the muscle system of the mantle of *B. cburneus*, same stain.

This may suggest that the maturation of the animal and of the cement gland elements may be synchronized with the molting cycle in the more primitive, but not in the more advanced balanids.

B. eburneus and *B. tintinnabulum* appear to be highly suitable for further demonstration, histochemically and ultrastructurally, of the relationship between the secretion granules and the nuclear and cytoplasmic constituents of the cement gland cell.

The role of the basal muscles, such as those seen in *B. eburneus*, *B. balanoides* and *B. amphitrite*, should be considered as an added mechanism for the distribution of cement to the basal plate region.

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SUMMARY

1. The histological characteristics of the cement gland cells of barnacles have been compared in *B. nubilis*, *B. psittacus*, *B. eburneus*, *B. balanoides* and *B. amphitrite*.

2. In *B. balanoides*, the cement gland cells show a very simple composition; they are situated at the base of the animal and the secretion appears scattered throughout the cytoplasm. The cement apparatus of *B. amphitrite* and *B. eburneus* looks like that of *B. tintinnabulum*, but in *B. psittacus* and *B. nubilis* the cement gland cells appear more complex.

3. The extrusion of the cement secretion is brought about by muscle fibers that pass through the connective tissue, and in *B. psittacus* and *B. nubilis* by elastic fibers around the secondary and principal canal systems.

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